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Effects of Administration Regime on the Psychotomimetic Properties of *d*-amphetamine in the Squirrel Monkey (*Saimiri sciureus*)

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SAMS-DODD, F. AND J. D. NEWMAN. *Effect of administration regime on the psychotomimetic properties of* d-*amphetamine in the squirrel monkey (*Saimiri sciureus*)*. PHARMACOL BIOCHEM BEHAV **56**(3) 471–480, 1997.—Studies have reported that *d*-amphetamine can induce a schizophreniform psychosis in humans and can induce abnormal behaviour patterns in monkeys that resemble the psychotic symptoms observed in man. The purpose of the present study was to identify a drug administration regime that in squirrel monkeys reliably could induce such behaviours in order to use this as a model of schizophrenia. The behavioural effects of acute, subchronic and continuous administration of *d*-amphetamine were determined in male and female squirrel monkeys during short term separation from the colony and in the home cage. It was found that abnormal behaviours developed in both male and female subjects and that they were most evident in the home cage. The number of subjects responding was highest during continuous infusion followed by subchronic treatment. The study indicated that prolonged administration of high doses of *d*-amphetamine is necessary for the development of abnormal behaviours. These findings suggest that animal models of schizophrenia based on *d*-amphetamine should be based on chronic administration or continuous infusion of *d*-amphetamine instead of acute injections. **Copyright © 1997 Elsevier Science Inc.**

Animal model Primates Psychosis Schizophrenia

AMPHETAMINE can induce a psychosis in humans that resembles paranoid schizophrenia. The psychosis normally occurs following a prolonged period (hours to days) of continuous abuse of high doses of amphetamine (1,3,4,14,18). The symptoms include paranoid delusions, ideas of reference, anxiety, stereotyped compulsive behaviour and visual and auditory hallucinations, and they occur in a state of clear consciousness and correct orientation (2,6,9,16,18).

This resemblance in many aspects between an amphetamine psychosis and paranoid schizophrenia has made *d*-amphetamine the primary model psychotomimetic agent in schizophrenia research (see 19 for a review). In primates, studies have shown that acute and subchronic administration of *d*-amphetamine cause hyperactivity, stereotyped behaviours, mouth and tongue protrusions and reduced affiliative behaviours between subjects (8,10,15,17,21,22,28), and that bizarre and hallucinatory behaviours and repetitive grooming behaviour develop following prolonged periods of *d*-amphetamine injections or continuous release of *d*-amphetamine from implanted pellets (13,20,26). These abnormal behaviours have been interpreted as delusions of parasitism, increased startle responses, fearfulness and social withdrawal, and have been suggested to represent a state in monkeys that correspond to an amphetamine psychosis in humans (13,20,25,26,28).

The results of these clinical and animal studies suggest that both dose level as well as temporal pattern of *d*-amphetamine administration affect the onset and degree of severity of a psychosis in humans and abnormal behaviour patterns in non-human primates. From the perspective of using *d*-amphetamine in monkeys as a model of schizophrenia it is primarily the abnormal behaviour patterns that are of interest, and it is therefore necessary to identify the administration regime

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TABLE 1

SUBJECTS AND THEIR TREATMENT DURING EACH PHASE OF THE STUDY

Name	Sex	Sub-species	Weight (g)	Acute (mg/kg)	Subchr. (mg/kg)	Continuous (mg/kg/h)
C006	Female	Roman	610	0.9	0.6	0.25
7793	Female	Roman	630	0.9	0.6	0.25
I4	Female	Gothic	650	0.6	0.6	0.25
I44	Female	Gothic	645	0.6	0.6	0.25
16A	Female	Roman	860	0.3	0.3	Vehicle
44A	Female	Gothic	800	0.3	0.3	Vehicle
G32	Male	Roman	780	0.9	0.6	0.25
G33	Male	Roman	770	0.9	0.6	0.25
I45	Male	Gothic	745	0.6	0.6	0.25
O2	Male	Roman	1000	0.6	0.6	0.25
W6	Male	Gothic	740	0.3	0.3	Vehicle
W10	Male	Gothic	875	0.3	0.3	Vehicle

of *d*-amphetamine that most reliably induces such behaviours in a majority of subjects. However, the relative advantage of one administration regime over another in terms of inducing these behaviours has not previously been established in a single group of animals, where such factors as individual variability in sensitivity and differences in methodology between laboratories can be controlled.

It is the purpose of the present study to compare the effects of acute, subchronic and continuous *d*-amphetamine administration on the behaviour of male and female squirrel monkeys. The behavioural effects of *d*-amphetamine are described in the separation test, where a subject is isolated from the colony for a short duration, in order to evaluate the anxiogenic action of *d*-amphetamine (24). In addition, observations of behaviour in the home cage, using focal observations of individual subjects following drug treatment, are conducted to determine the effects of *d*-amphetamine on the behavioural repertoire. Based on these results we will first address the question of how drug administration regime affects the development of abnormal psychotic-like behaviours; secondly, we examine whether male and female subjects respond differently to the effects of *d*-amphetamine; and thirdly, the test models are compared and their ability to detect *d*-amphetamine-induced behavioural changes are evaluated.

METHODS

Subjects

Six adult male and six adult female squirrel monkeys (*Saimiri sciureus*) were used for this study (Table 1). They were housed as same-sex pairs in cages (w,d,h: 0.61 × 0.61 × 1.52 m) and were part of a larger colony, permitting auditory, visual and limited tactile contact with other conspecifics. Colony members were maintained on a normal 12-h light/dark cycle, and were provided with ad libitum water, twice daily rations of Purina New World Monkey Chow, and twice weekly rations of fresh fruit. Subjects had previously been given low doses of yohimbine HCl and benactyzine HCl, but had been drug-free for more than two years.

Drug Treatment

d-amphetamine sulphate (*d*-amphetamine · 1/2 sulphate MW = 184.3; Nomeco, Copenhagen) dissolved in 0.45% sterile saline and vehicle solution (0.45% saline) were filtered using Millex-HA 0.2 μm filter units (Millipore Corp.). For acute and subchronic drug regimes intramuscular injections (volume of 0.1 ml/kg) were given in the thigh. Continuous, subcutaneous infusion of *d*-amphetamine or vehicle were given for 6 days by osmotic minipumps (model 2ML2, Alzet Corp.) with a pumping rate of 4.99 ± 0.21 μl/h (mean ± sd, specified by manufacturer). Prior to minipump implantation each subject was anaesthetised with an initial dose of 15 mg/kg IM injection of Ketamine (Ketaset®, Fort Dodge Labs). Supplementary doses of 5 mg/kg IM were given if necessary. Ketamine is a NMDA-antagonist and has a very different receptor profile than *d*-amphetamine. It was selected to avoid any interactions with *d*-amphetamine. The pump was implanted under sterile conditions beneath the skin on the back of the monkey. The wound was sutured, sprayed with antibiotics (Furazolidone®, Veterinary Products) and the monkey received an injection of 60,000 IU penicillin. It quickly recovered and was returned to its home cage. In approximately 1–2 h effects on subjects receiving *d*-amphetamine could be observed. The pumps were removed after 7 days using the same procedure as above. All subjects recovered completely after the study.

Separation Test

The subject was placed inside a wire-mesh test cage (1 m^3) in a separate room away from the colony, but allowing some audible contact. Behaviours and vocalisations were recorded continuously by a closed-circuit videosystem. The spontaneous behaviour was recorded for 15 min in the test cage, whereafter the subject was returned to its home cage. Tests were conducted between 10.00 h and 13.00 h with a one week interval to avoid habituation to the separation procedure (24). The test cage was not cleaned between trials in order to provide a constant odour level in the cage. This may have left olfactory cues in the cage that affected the behaviour of the next monkey being tested, but this procedure was chosen because it was impossible to adequately clean the cage between trials, and because any single cue would have been masked by the presence of other cues.

The tapes were scored for frequency and duration of locomotor activity (walking, running, climbing and jumping) and frequency of different vocalisations (categorised according to (23) including isolation peeps, alarm calls and chucks.

Home Cage Behaviour

Focal observations of behaviour were made in the home cage between 14.00 h and 18.00 h. A single trained observer sat in front of the cage at a distance of approximately 2 meters. The monkeys were habituated to the presence of the observer before the actual experiments began and the same observer was used throughout this study for the behavioural analysis. The observer was not blind to the treatment paradigm. Each subject was videofilmed and observed continuously for 10 min using on-line registration on a transportable computer with The Observer (Noldus Information Technologies, b.v.). The total frequency and duration of the following behaviours were recorded:

General activity: The subject is moving around in the cage in a non-stereotyped manner.

Sleep: Subject is crouched up and is sitting quietly.

Grooming: Cleaning its own fur or scratching itself.

Cage stereotypy: Movement patterns along particular routes in the cage. These behaviours are present independently of drug treatments and have developed as a consequence of being in captivity. They usually have a particular appearance for each individual.

Amphetamine stereotypy: Stereotyped postures or movements that have developed after administration of *d*-amphetamine and that are different from the cage stereotypy, e.g., repetitive grooming movements, rocking back and forth, small head movements or tongue protrusions. These postures showed great variation between subjects, but each subject generally displayed the same behaviour in response to *d*-amphetamine.

Huddle: The two subjects in a cage are sitting close together and are touching.

The frequency of the following parameters was also recorded:

Eat: The subject places food into its mouth.

Vocalisations: Alarm calls, isolation peeps, twitters, chucks and a category of other calls were scored (23).

In addition, aggressive behaviour, mounting and shaking of cage were included in the observations, but they were not seen during the experiments and are therefore excluded hereafter.

Experimental Procedure

The testing procedures were standardised with separation tests being performed in the morning and observations of home cage behaviour in the afternoon. Subjects in the same cage always received identical treatments. All the females were tested on one day and males on the following day. The monkeys were always tested and observed in the same order. For experiments involving injections of *d*-amphetamine or vehicle each subject would receive an injection 30 min before testing in each test, thus receiving 2 injections per day of the same dose with a minimum interval of 5 h (range 5 to 6 h) on the days of behavioural observations; otherwise the minimum interval was 4 h (usually 5–6 h). Each subject always received the same dose throughout each phase of the study.

The study consisted of 4 phases (Tables 1 and 2). In the first phase on Day 1, a dose-response curve to *d*-amphetamine was established for the separation test and the home cage behaviour paradigm with 3 doses of *d*-amphetamine (0.3; 0.6; 0.9 mg/kg) including 2 male and 2 female subjects per dose. The dose regime was based on previous findings (22). These authors found strong effects of 0.3 and 0.6 mg/kg IM *d*-amphetamine on stereotyped and social behaviour in the squirrel monkey. The upper dose of 0.9 mg/kg was included to verify that no additional advantages were gained by increasing the dose. However, at 0.9 mg/kg the *d*-amphetamine-induced head movements were very pronounced and signs of repetitive motor patterns were observed. We did not want to include dose levels that impaired the locomotor abilities of the subjects. Clinical studies of the amphetamine psychosis in humans (see introduction) have established that a psychosis can develop at dose levels below those that cause motor disturbances. Therefore, the highest dose was omitted during subchronic administration.

Next, the subjects were given 3 days to recover and on Day 5 the second phase with subchronic treatments of either 0.3 or 0.6 mg/kg *d*-amphetamine began. Each subject would receive 2 daily injections (morning and afternoon) of a fixed dose for 4 days (Day 5–8). The subjects were observed in the home cage on Day 5 and 8, and in the separation test on Day 8. In the period Day 12 to 15 all subjects received subchronic vehicle treatments following the same schedule as during the subchronic *d*-amphetamine study. The vehicle treatment regime was placed between the subcutaneous and continuous *d*-amphetamine dose regimes in order to establish a control level for a group of subjects that had been exposed to *d*-amphetamine rather than to use a completely drug-naive group as *d*-amphetamine can induce chronic changes in behaviour.

In the final phase osmotic minipumps were filled with either vehicle or 0.25 mg/kg/h *d*-amphetamine and were implanted subcutaneously on Day 19 in all subjects. The dose selection was based on observations during the subchronic dose regime that a single injection of 0.6 mg/kg would visibly affect the behaviour of different subjects for about 3 h. Considering that some tolerance would develop and that during infusion continuous breakdown of *d*-amphetamine would occur, we chose a dose of 0.25 mg/kg/h. Observations of home cage behaviour were conducted on Day 20, 22, and 24 and the subjects were tested in the separation test on Day 23. Pumps were removed on Day 26 and the subjects were allowed to recover.

Data Analysis and Statistics

Behavioural data were analysed with The Observer® version 2.0 or a Beta release of version 3.0 (Noldus Information Technologies, b.v.) and are shown as individual data points in all figures to demonstrate the range of variability between individual subjects. In addition the mean response is indicated. Locomotor activity and number of isolation peeps in the separation test are given as percent of vehicle level (result divided by vehicle (Day 15) multiplied by 100), thereby showing the relative change in behaviour as a result of drug treatment. Data for stereotyped behaviour in the separation test and observations on home cage behaviour are given as total duration (sec) or frequency during an observation period. The vehicle response on Day 15 after a 7 day wash-out period was used as control level for the statistical analysis.

All statistical analyses were performed on actual data values. For the separation test General Linear Modelling was used. For comparison across dose levels tested in the home cage Kruskal-Wallis one-way analysis of variance was used, and for repeated measurements of subjects at a particular dose level or treatment Wilcoxon signed ranks test or Friedman two-way analysis of variance were used (Systat 5.2 software).

TABLE 2
DESIGN OF STUDY

Day	Treatment	Dose of *d*-amphetamine and Subjects per Dose*	Isol. Test (Day)	Home Cage (Day)
1	Acute	0.3 mg/kg (2M + 2F); 0.6 mg/kg (2M + 2F); 0.9 mg/kg (2M + 2F);	1	1
5–8	Subchronic	0.3 mg/kg (2M + 2F); 0.6 mg/kg (4M + 4F);	8	5, 8
12–15	Subchronic	Vehicle (6M + 6F);	15	12, 15
19–26	Continuous	Vehicle (2M + 2F); 0.25 mg/kg/h (4M + 4F);	23	20, 22, 24

*Abbreviations: (M): male; (F): female.

The abnormal behaviour patterns reported for different individuals during the experiments were verified by subsequent viewing of all the videotapes by a different observer blind to the treatment than the person that had performed the on-line behavioural analysis. This was done to ensure that the descriptions were correct.

RESULTS

Separation Test

Most of the monkeys displayed a high activity level in the separation test after vehicle treatment. They emitted many isolation peeps and appeared distressed, and a few subjects also showed some cage stereotyped behaviour. After acute and subchronic administration of *d*-amphetamine neither males nor females responded strongly to the separation from the colony (Fig. 1). *d*-amphetamine induced dose-dependent decreases in both the locomotor activity level and the number of isolation peeps following acute ($P < 0.05$ and $P < 0.05$, respectively) and subchronic ($P < 0.001$ and $P < 0.01$, respectively) administration. However, the subjects remained vigilant at all dose levels in spite of the reduced locomotor activity. This was evident by the lack of direct motor disturbances and by how the subjects actively inspected the room from their position in the cage. *d*-amphetamine also induced very pronounced head movements and signs of repetitive motor patterns at 0.9 mg/kg, whereas the lower dosages only induced slight repetitive head movements, that were insufficient to interfere with the locomotor activity and ability to vocalise (data not shown). The monkeys did not show any abnormal behaviours, e.g., staring, during the acute and subchronic administration regimes.

Next, the response to four days of continuous administration of 0.25 mg/kg/h *d*-amphetamine or vehicle was compared to subchronic vehicle injections (Fig. 1). The activity level and number of isolation peeps for the group receiving vehicle pumps were unaffected as was the general behavioural response to separation when compared to subchronic vehicle injections. In the amphetamine-treated group a significant decrease was seen in locomotor activity ($P < 0.01$). The number of isolation peeps was also affected ($P < 0.05$), but the response was very variable and ranged from a 350% increase to a total abolishment. The subjects only displayed very slight or no amphetamine-induced head movements. Also no motor disturbances were seen due to the treatment and they remained vigilant. All subjects, except I44, displayed a staring behaviour in which they would fixate on a particular spot on the floor or on the wall and would for several seconds stare in this direction. Some subjects also appeared very distressed and fearful by the separation procedure. Others performed complex stereotyped behaviours such as continuously grooming the fur, while making isolation peeps and chucks or were continuously moving their hands (like piano-playing).

Home Cage Behaviour

Observations of behaviour in the home cage were conducted in the afternoon 1 to 2 h after the subjects were fed. The normal behaviour pattern after vehicle injections was looking for food, eating, grooming and sleeping. Social interactions were frequent, but the levels of social grooming and huddle were generally low and were almost absent between males.

Following acute injections of *d*-amphetamine the normal pattern and frequency of these behaviours were strongly affected in males and females (Fig. 2). The acute treatments caused a significant effect on activity ($P < 0.05$) with a decreased activity level at 0.6 mg/kg and an increase at 0.9 mg/kg. *d*-amphetamine also caused dose-dependent decreases in grooming ($P < 0.001$), eating ($P < 0.01$) and number of vocalisations ($P < 0.05$), and huddle was completely disrupted in the cages where it normally was present. Acute *d*-amphetamine generally did not affect the level of cage stereotypy, but did induce a dose-dependent increase in the level of amphetamine-induced stereotyped behaviours ($P < 0.01$). The stereotyped behaviours will be treated in detail below.

The effects of subchronic *d*-amphetamine or vehicle treatment for 4 days with behavioural observations in the home cage on the last day of treatment, i.e. Day 8 and Day 15, were similar to the effects of acute *d*-amphetamine treatment in most respects (Fig. 3). The levels of locomotor activity and cage stereotyped behaviour were unaffected by the subchronic treatment in both males and females, while grooming ($P < 0.01$), eating ($P < 0.001$) and number of vocalisations ($P < 0.01$) were dose-dependently decreased. The level of amphetamine-induced stereotyped behaviour was also significantly increased ($P < 0.01$) and now, unlike acute treatment, included abnormal behaviour patterns. As with acute treatment huddle was strongly reduced and some subjects actively avoided contact with conspecifics.

A comparison between first and last day of subchronic *d*-amphetamine treatment (Day 5 and 8) demonstrated that there were no additional effects of the repeated treatment regime on most of the behavioural categories. However, the level of cage stereotypy was significantly higher ($P < 0.05$)

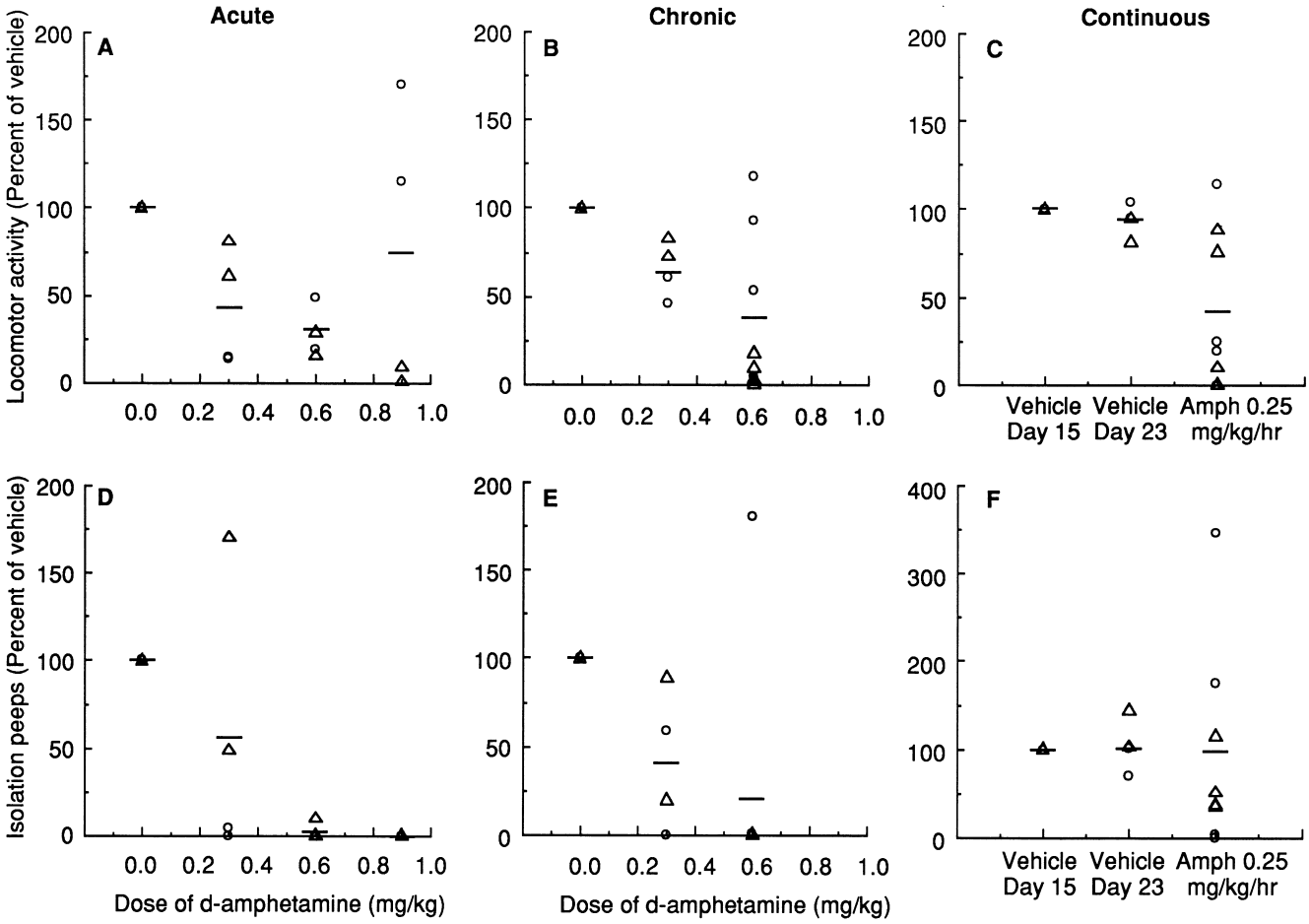

FIG. 1. Effects of acute, subchronic and continuous *d*-amphetamine on the behavioural response in the separation test (Day 1, 8, 15 and 23). In A, B, C level of locomotor activity; and in D, E, F number of isolation peeps. The behavioural response of the individual subjects is expressed as a percentage of their response to vehicle treatment (Day 15). This vehicle response (mean ± SEM) was for locomotor activity: males (512.1 ± 79.9 sec.; range 247 to 760 sec.) and for females (474.4 ± 113.6 sec.; range 162 to 823 sec.); and for number of isolation peeps: males (180.5 ± 48.9 peeps; range 80 to 356 peeps); and females (107.8 ± 35.4 peeps; range 13 to 246 peeps). Symbols: males (△); females (○); and the mean response for each dose/time point (–).

on the first day compared to the last day of treatment, and the appearance of the *d*-amphetamine-induced stereotyped behaviours had changed.

Observations of home cage behaviour during continuous infusion with minipumps demonstrated that the vehicle group was very stable in its response during the infusion period, whereas the *d*-amphetamine-treated monkeys markedly changed their behaviour (Fig. 4). Comparison across the observation period, Day 15 to Day 24, showed that *d*-amphetamine significantly reduced the level of cage stereotypy ($P < 0.01$), while amphetamine stereotyped behaviour ($P < 0.001$) and huddle ($P < 0.05$, data not shown) were significantly increased. The activity level, grooming and eating behaviours were not affected.

Comparison within the amphetamine group across Day 20–24 only revealed a significant increase in the level of eating ($P < 0.05$). As with acute and subchronic treatment reduced eating behaviour occurred after implantation of the pumps, but it returned to baseline level after the first day. There were also tendencies toward increased levels of activity ($P = 0.09$) and grooming ($P = 0.07$), possibly as a result of the implanted pumps, whereas the levels of cage and *d*-amphetamine stereotyped behaviour were unaffected.

Stereotyped Behaviour Patterns

The stereotyped behaviour patterns could be divided into (i) a cage stereotyped behaviour and (ii) a *d*-amphetamine stereotyped behaviour that again could be separated into an invariant form that included head movements and tongue protusions, and a more variable form that included the abnormal behaviour patterns. The *d*-amphetamine treatments caused increases in the frequency of cage stereotyped behaviour in some subjects, but these effects were variable and inconsistent. The amphetamine-induced stereotyped head movements and tongue protrusions on the other hand occurred in a dose-dependent fashion in most subjects, and these behaviours were invariant between subjects. These behaviours were present during acute and subchronic administration of *d*-amphetamine and on the first 1 to 3 days of continuous infusion. The subjects appeared to develop tolerance to this effect of *d*-amphetamine with time, and the head movements and tongue protrusions

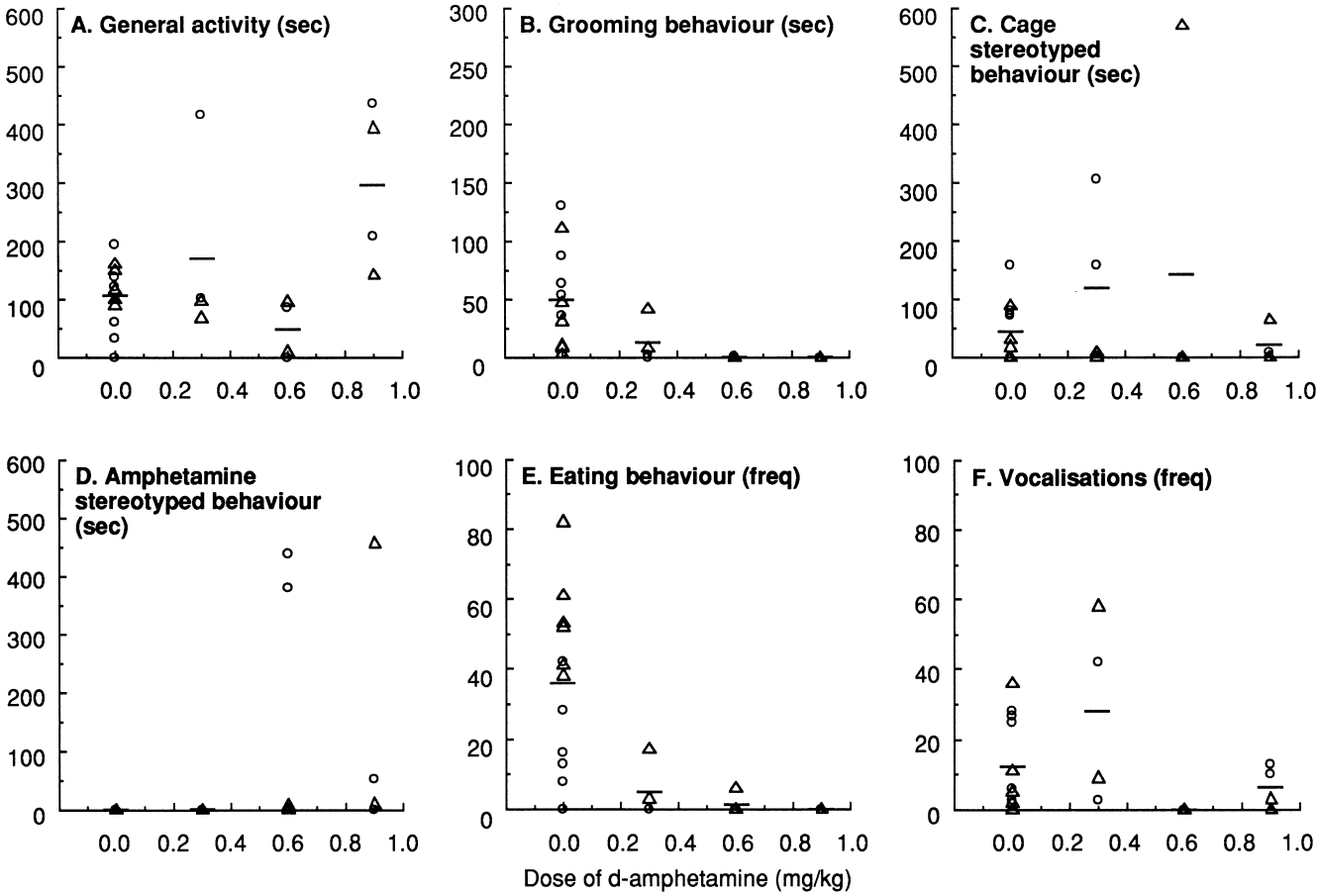

FIG. 2. Effects of acute amphetamine on behaviour in the home cage (Day 1 and 15). Symbols as in Fig. 1.

disappeared during the final phase of the continuous infusion. *d*-amphetamine also induced different abnormal behaviour patterns and these were most evident in the home cage. The abnormal behaviours demonstrated considerable variation between subjects, but were relatively stable for a given subject once they had developed. Each subject would display several symptoms, but the composition of the behaviour could change from one moment to the next in some subjects or remain very stable for extended periods in others. Usually the subjects were completely engaged in these behaviours and often did not respond to noises or rapid movements made close to the cage. The abnormal behaviours included orienting responses towards empty points in space; grasping into empty space; picking and grooming the skin, and brushing legs and feet with hands as if the subject was removing objects; startle responses, where the subject would suddenly move from one place to another; sitting in a corner looking into the room with fearful expression; and staring behaviour, where the subject would fixate for prolonged periods on particular spots. They contained a strong element of stereotypy within them, but these behaviours were more complex than the repetitive head movements and tongue protrusions observed following acute and subchronic doses of *d*-amphetamine.

The abnormal behaviour patterns demonstrated a different temporal pattern in their development than the acute onset of repetitive head movements and tongue protrusions following *d*-amphetamine administration. In Table 3 the number of subjects that displayed the abnormal behaviours have been summarised for different stages of each administration regime and for different dosages. The data demonstrated that development of these behaviours was dose-dependent, had a delayed onset and that they were observed in an increasing number of subjects as the duration of drug administration increased.

DISCUSSION

Behavioural Effects of d-*amphetamine*

The present study has compared the effects of different dosage and administration regimes of *d*-amphetamine on the behaviour of male and female squirrel monkeys. To briefly summarise, acute and subchronic *d*-amphetamine administration caused dose-dependent decreases in the frequency and duration of simple and complex behaviours in both male and female squirrel monkeys. Behavioural elements such as vocalisation rates in the separation test and grooming and eating in the home cage were reduced as well as the level of huddle, where some subjects actively avoided contact with the cage mate. In addition, acute and subchronic administration increased the frequency of stereotyped behaviour such as head movements and tongue protrusions, while the abnormal behaviours were mainly present during the last part of the subchronic dose regime.

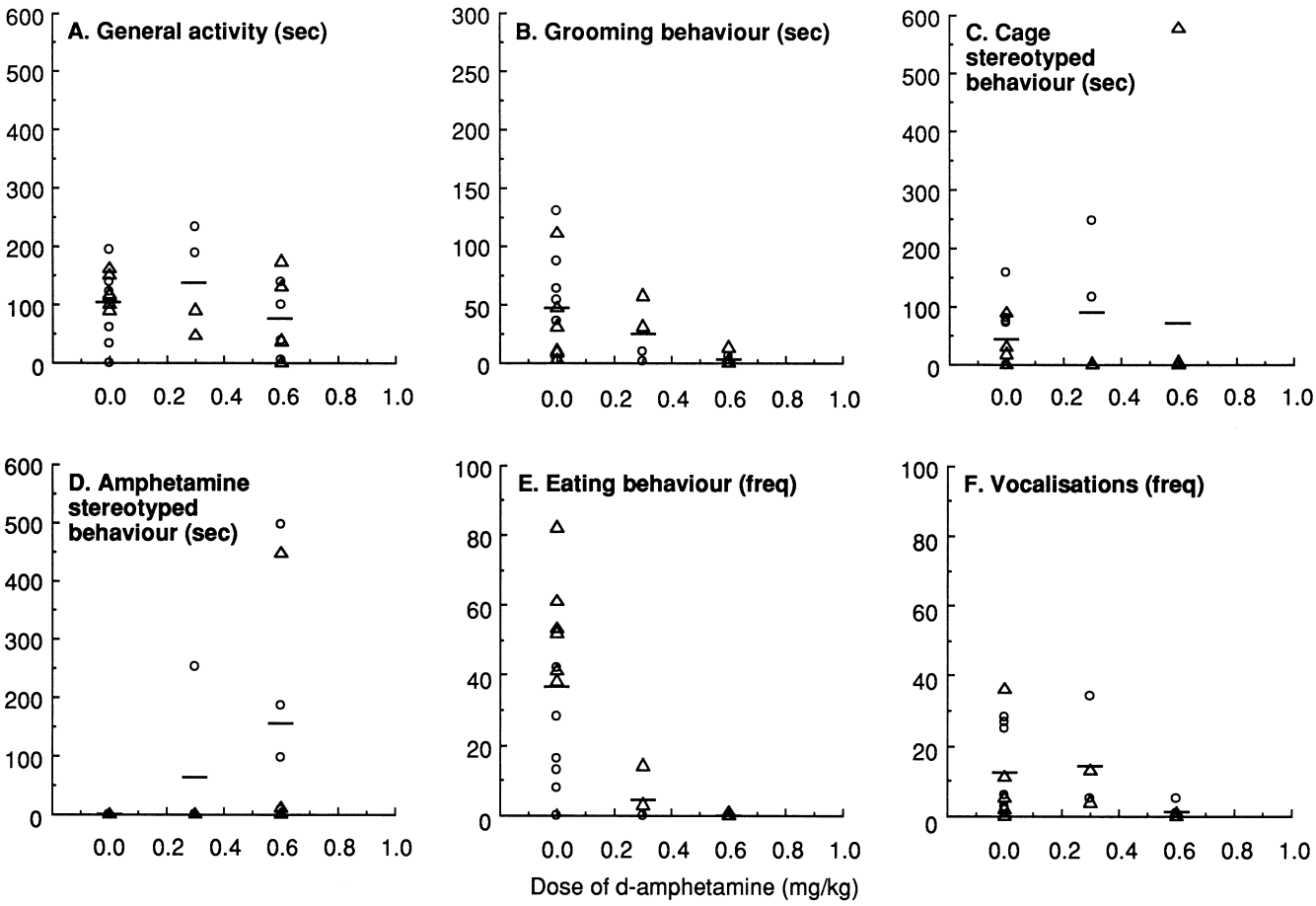

FIG. 3. Effects of subchronic amphetamine on behaviour in the home cage (Day 8 and 15). Symbols as in Fig. 1.

The continuous drug regime had a very different effect on these behaviours in both sexes. The frequency and duration of eating and grooming were not reduced, except for a transient decrease in eating on the first day following implantation, but instead remained on a level similar to vehicle-treated monkeys. The effect on social behaviours was less clear as the level of huddle actually increased in some cases, and the calling rates in the separation test and the home cage were very variable and did not display the same consistent decreases as were observed during acute and subchronic administration. The abnormal behaviours were displayed vividly throughout the implantation period, particularly in the home cage, and in some cases they tended to increase in severity with time. In contrast, the more invariant stereotyped motor patterns such as head movements and tongue protrusions showed tolerance and disappeared after 1 to 2 days of infusion.

The restricted access to primates for such studies often place restrictions on study design and can necessitate a less than optimal experimental protocol. The present study has described the effects of administration regime on the psychotomimetic properties of *d*-amphetamine in a single group of squirrel monkeys. The use of the same subjects throughout the study has the advantage that individual differences between subjects is reduced, but it also has the disadvantage that the same subject is repeatedly exposed to *d*-amphetamine over a short time period. This is particular evident for the selection of baseline level for the different behavioural tests. In the present design the vehicle treatment was placed after subchronic *d*-amphetamine injections following a wash-out period of one week, but it is possible that this was too short a period since *d*-amphetamine can have long-term effects. This can introduce a potential error when measuring the specific behavioural parameters in the separation test and in the home cage, whereas the effect on abnormal behaviour patterns will be minimal as these disappeared fairly rapidly following cessation of *d*-amphetamine administration. However, the extent of this error is probably limited as the baseline level between the last day of vehicle injections and the following days of vehicle infusion was very stable. Also, the main effect of scoring vehicle trials before all the amphetamine effects wore off would be to reduce the difference between vehicle and drug behaviours and therefore the amphetamine-induced behaviours that are reported here may actually be underestimated. However, a follow-up study to evaluate these effects that include baseline observations before and after drug treatment is necessary to address this issue.

Another issue that needs to be addressed is the selection of dose during the continuous infusion of *d*-amphetamine. The present dose was selected on basis of observations on the effects of subchronic *d*-amphetamine and the duration of action following a single injection. However, from these data it is not possible to determine the steady-state dose that the

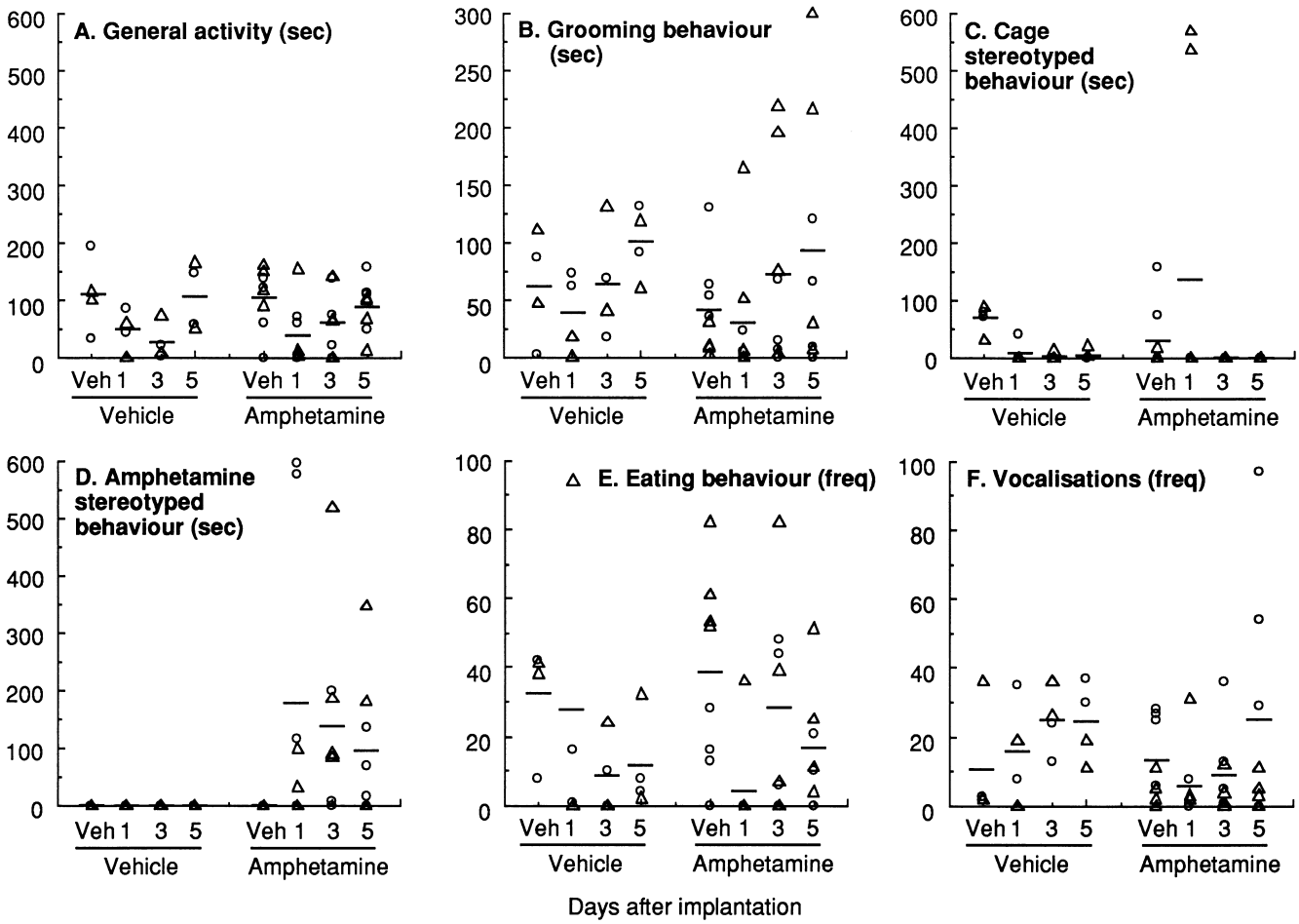

FIG. 4. Effects of continuous infusion of *d*-amphetamine on behaviour in the home cage (Day 15 and 20-24). Symbols as in Fig. 1.

monkeys actually received and it is therefore necessary to make a more complete comparison by determining a dose-response curve for continuous *d*-amphetamine infusion. Indeed, the present data indicate that such a study is justified and that it can provide important results on the psychotomimetic effects of *d*-amphetamine.

In spite of these possible sources of error, the results of the present study appear very similar to previously reported findings in primates. Acute and subchronic *d*-amphetamine induced hyperactivity, stereotyped behaviours, mouth and tongue protrusions comparable to previously reported findings, and subchronic and continuous *d*-amphetamine administration induced the same abnormal behaviour patterns that in previous studies have been compared to an amphetamine psychosis in man (13,20,25,26,28). The present study found no clear sex differences in the response of male and female squirrel monkeys to acute and subchronic *d*-amphetamine for most of the behavioural parameters, which correspond well with previous findings (22). However, some indication of sex differences were observed with respect to cage stereotyped behaviour and grooming. These findings in squirrel monkeys also correlate well with clinical studies that have shown that male and female volunteers respond similarly to the effects of *d*-amphetamine and are approximately of the same risk of developing an amphetamine psychosis (5,11,12). Overall, the present study has been able to replicate the findings of these previous studies.

Effect of Administration Regime

The purpose of the present study was to determine an administration regime that was optimal for inducing a psychotic state in monkeys that corresponded to a *d*-amphetamine psychosis in humans. The study demonstrated that the development of these abnormal behaviours in male and female monkeys depended strongly on both dose and duration of drug administration, and that there was a clear positive correlation between the duration of *d*-amphetamine administration and the number of monkeys that developed these behaviours (Table 3). Clinical studies have shown that in humans a *d*-amphetamine psychosis is only precipitated following long-term drug administration and at relative high dosages (1,3,14,18). Several other animal studies have likewise demonstrated that prolonged or continuous treatment with *d*-amphetamine can result in the development of abnormal behaviours (13,20,25, 26,28). Thus the consensus of all of these findings is that chronic or continuous administration of drug for at least three days are necessary in order to precipitate abnormal behaviours in a majority of animal subjects.

Evaluation of Behavioural Models

The separation test was originally developed as an anxiogenic model and is sensitive to anxiolytic compounds (24). *d*-amphetamine induced dose-dependent decreases in the

TABLE 3

NUMBER OF SUBJECTS DEVELOPING ABNORMAL BEHAVIOUR PATTERNS IN RESPONSE TO *d*-AMPHETAMINE DURING THE DIFFERENT ADMINISTRATION REGIMES

Dose mg/kg	Acute	Subchronic (Day 1)	Subchronic (Day 4)	Continuous (Day 2)	Continuous (Day 5)
0.0		0 (n = 12)	0 (n = 12)	0 (n = 4)	0 (n =4)
0.3	0 (n = 4)	0 (n = 4)	1 (n = 4)		
0.6	1 (n = 4)	2 (n = 8)	4 (n = 8)		
0.9	1 (n = 4)				
0.25/hr				7 (n = 8)	6 (n = 8)

number of isolation peeps, which is indicative of an anxiolytic effect, but this seems puzzling, since *d*-amphetamine clinically does not have any anxiolytic properties. Studies of another putative anxiogenic compound, benactyzine HCl, likewise resulted in a dose-dependent decrease in vocalisations during the separation test (7). However, in that study drug-related increases in alarm calls during a challenge procedure were also reported. A test for inducing alarm behaviour was not incorporated into the present study, but the reduction in isolation peeps that occurred in some animals with amphetamine administration may be a reflection of increased fear or alarm, as suggested by the fearful glances observed in some subjects during the separation test. With respect to the abnormal behaviours they were almost absent in the separation test during acute and subchronic administration and were during continuous administration much less severe than those observed in the home cage. Overall these findings indicate that the separation test is not particularly well suited for use with psychotomimetic compounds.

In the home cage acute and subchronic *d*-amphetamine administration caused dose-dependent changes in self-grooming, eating, vocalisations, huddle, and stereotyped behaviours. The treatment changed the entire structure of the subjects behaviour in fairly easily quantifiable ways. In addition, during the different administration regimes, the appearance of abnormal behaviour patterns (Table 3) were easily recognizable. The social contexts thus appeared to reveal more abnormal behaviours compared to the separation test, and this suggests that observations of home cage behaviour is the most appropriate behavioural test for measuring the psychotomimetic properties of *d*-amphetamine.

CONCLUSION

The primary purpose of the present study was to describe the ability of different administration regimes to induce the development of abnormal behaviour patterns in squirrel monkeys. These behaviours may be closely related to the psychotic symptoms that are observed during a *d*-amphetamine psychosis in man and are therefore important when attempting to model schizophrenia in animals. The effects of acute, subchronic and continuous *d*-amphetamine administration were studied in a single group of monkeys in order to reduce the effects of individual variations in sensitivity. Based on these findings it was found that chronic or continuous drug administration were necessary in order to reliably induce the behaviour patterns that correspond to psychotic symptoms in man. This finding corresponds well with previous findings in primates (13,20,26,28) and raises the possibility that the effects of acute vs. long-term effects of *d*-amphetamine on the nervous system may involve different neural mechanisms. If this is correct, it necessarily implies that studies of the acute effects of *d*-amphetamine may not be related to the psychotomimetic properties of *d*-amphetamine and that acute treatment may not be a valid model of schizophrenia (a concern previously raised by 13, 26). The findings of this study therefore suggest, that the clinical situation should be more closely mimicked in animal models of schizophrenia in order to ensure their validity.

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